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Interactive comment on "Vertical profiles of sediment methanogenic potential and communities in two plateau freshwater lakes" *by* Yuyin Yang et al.

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Response to Referee #2's comments 1) General comments This manuscript describes and compares methanogenesis in sediments from a eutrophic and a mesotrophic lake, with a focus on methanogens potential activity, abundance and diversity along vertical profiles. The results presented here are of interest. However, this manuscript could be significantly improved. Indeed, the current version suffers from (i) a lack of general conclusion (i.e. the discussion and conclusion are superficial), (ii) a writing style that is sometimes confusing for the reader, (iii) poor-quality figures, and (iv) a lack of geochemical background. Concerning this last point, the geochemical background is available in the SI but totally absent from the main text and the discussion. This in-

formation could significantly help the discussion and the interpretation of the results. Response: The authors appreciate the reviewer's valuable comments. In the revised manuscript, (i) General conclusion has been added to the manuscript, (ii) writing has been improved, (iii) some figures have been redrawn as suggested, (iv) geochemical background of the two lakes has been added to the main text.

2) Specific comments a. Abstract - l. 25-26: "changes of methanogens". Do you mean change in abundance or taxonomic change? Please be more specific. Response: The authors appreciate the reviewer's suggestions. The revision has been made as follows: "knowledge on the layer depth-related changes of methanogen community structure and activities in freshwater lake sediment. . ."

- l. 35-36: For each lake, specify if it is mesotrophic or eutrophic. Response: The revision has been made as follows: "The layer depth-related change pattern of the methanogenesis potential in eutrophic Dianchi Lake was found to be different from that in mesotrophic Erhai Lake."

- l.33-43: This second part of the abstract looks like a list of results. It would be nice to have here some concluding remarks, i.e. put these results in the context of what we know about methanogenesis in these environments. Response: The authors appreciate the reviewer's valuable suggestions. The revision has been made as suggested.

- l.46-47: I am not sure that it makes sense to choose keywords that already appear in the title and/or in the abstract. Response: The authors appreciate the reviewer's suggestions. 'vertical profiles' has been removed from the keywords, and 'trophic status' has been added.

b. Introduction - l. 77-78: This short description of methanogenesis pathways is oversimplified. Please expand or at least specify that those are the main pathways (and not the only ones). Response: The authors appreciate the reviewer's suggestions. The revision has been made as follows: In freshwater lake, organic matter is fermented to acetate, CO2 and H2, which are further converted to CH4 by methanogens. There

are two major pathways: hydrogenotrophic pathway (using H2/CO2) and acetoclastic pathway (using acetate, i.e. the methyl group) (Conrad et al., 2010). And the relative contribution of the two pathways varies in different lakes (Conrad, 1999).

- l. 81: What do you mean here by "theoretical ratio". Do you mean that we consider that, in most of natural environments, 2 molecules of CH4 are produced by acetoclastic methanogen per molecule of CH4 produced by hydrogenotrophic methanogens? Please be more specific. Response: The authors appreciate the reviewer's suggestions. The revision has been made as follows: Despite the theoretical ratio of 2:1 (acetoclastic pathway: hydrogenotrophic pathway) when carbohydrates or other similar form of organic matter was degraded. . .

- l.85-86: Does the 16S or mcrA sequencing give some insights about the methanogenesis pathway? Response: Yes. Certain genus of methanogens might be related to certain pathway. For instance, the order Methanomicrobiales dominated in most samples, and it was hydrogenotrophic.

- l. 93: What is a humic lake? Could you please define it? Response: In the reference, it generally refers to a lake which contains darkly stained acid water.

- l. 96: What do you mean by substrate here? Are you talking about the source of electron (hydrogen, acetate)? Response: Organic matter (the same as mentioned in the references).

c. Materials and methods - l. 108-109: Here we are missing some background information about these 2 lakes. I propose the following: Indicate where these 2 lakes or located on a map, and describe them by listing some key features (that make these lakes eutrophic or mesotrophic, and/or that are relevant for this study). This information could be included in the figure containing the map. Response: The authors appreciate the reviewer's valuable suggestion. A table has been added to provide essential background.

- l. 113-114: Is this information relevant to the study? Response: Yes. The incubation temperature was set close to the in-situ temperature.

- l. 120: The physicochemical analyses are simply absent from the manuscript. They should be included in the main text and more importantly in the discussion. Response: The authors appreciate the reviewer's suggestions. The physicochemical characteristics of the two lakes have been well studied in a series of articles (e.g. a 26-article thematic issue on Environmental Earth Sciences, volume 74, issue 5). So here we just provide a general background of Dianchi Lake and Erhai Lake. We have added some key points as suggested. The methanogen community structure and activity are always thought to be related physicochemical parameters of course. However, in vertical profiles, both physicochemical parameters (e.g. DOC, TN, ORP) and microbial community change with depth. The co-variation makes correlation analysis doubtable. That is to say, though we observed significant positive correlation between TMP and sediment NH4+-N, TP and TOC in our study, we could not draw a conclusion that TMP was impacted by these parameters. There still remains possibility that depth has similar impact on TMP and these parameters so they just show a similar change pattern.

- l. 125-127: At this point, we don't want to know where to find the results, but how did you proceed to obtain these results. Response: The authors appreciate the reviewer's suggestions. The revision has been made as suggested.

- l. 137: What is the final CH3F concentration in the incubations? Response: 2%.

- l. 130-141: How did you calculated the rates from these incubations? Response: In the new version of manuscript, the rate has been replaced by total methane production.

- l. 144-161: What is the qPCR efficiency and how was inhibition tested? Response: qPCR efficiency was 96.52% for Archaeal 16S rRNA gene, and 92.93% for mcrA gene. Inhibition was tested using Cq dilution method.

- l. 166: What kind of triplicates are you talking about? Biological, technical? Re-

sponse: Biological.

- l. 170-171: “After subsampling to the lowest number of sequences”. What do you mean here? Response: Since different numbers of sequences were obtained from NGS, calculating the diversity index (maybe as well as some other parameters) using all the raw data might result in bias (i.e. higher number of sequences might results in higher diversity). So sequences in each sample were random sampled to a same number (to make each sample contain same number of sequences).

- l. 163-176: Did you check your sequences for chimeras? Response: Sorry that we made a mistake at line 180. We checked our archaeal 16S rRNA sequences (instead of mcrA sequences) for chimeras during the cluster process.

- l. 174-176: Which method did you use for taxonomic annotation (Silva is only a database) Response: Naive Bayesian classifier (http://sourceforge.net/projects/rdp-classifier/).

- l. 180: Which method did you use for detecting chimeras? Response: Sorry that we made a mistake at line 180. We checked our archaeal 16S rRNA sequences (instead of mcrA sequences) for chimeras during the cluster process.

- l. 186-187: The PCoA and environment clusters analysis are missing from the manuscript. Response: The revision has been made as follows: . . .and cluster analysis were conducted based on Weighted Unifrac distance. . . The result was shown in figure 5.

d. Results - l. 195: I guess DW stands for dry weight. You should mention in the methods that the rates were calculated relative to the dry weight of sediments (and how you measured the dry weight of the sediments). Response: The authors appreciate the reviewer’s suggestions. The revision has been made as follows: (DW stands for dry weight, which was determined gravimetrically). . .

- l. 201: I think you mean HMP, not MPP. Response: The authors appreciate the

reviewer’s suggestions. The revision has been made.

- l. 204-207: It would be nice to see these results. A plot including MPP, AMP and HMP would help the reader to understand the results. Response: The authors appreciate the reviewer’s suggestions. The revision has been made.

- l. 226: What is this normalization? Response: In the new version, it has been replaced by subsampling (as is described in line 170-171).

- l. 228: What is exactly the library coverage? I guess it’s the proportion of 16S from the community that was sequenced. How was it calculated? Response: Library coverage is an estimation of the proportion of genes from the community that was sequenced. And it was calculated as C=1-n/N, where n is the number of OTUs without a replicate, and N is the total number of sequences.

- l. 251: If you use this annotation, you should define it somewhere (in the Methods). Response: The authors appreciate the reviewer’s suggestions. The revision has been made as: The five replicate sediment cores were sliced into the layers (sample D1 or E1:0–5 cm, sample D2 or E2:5–8 cm, sample D3 or E3:8–11 cm, sample D4 or E4:11–14 cm, and sample D5 or E5:14–17 cm, sample D6 or E6:17–20 cm). Samples D1–D6 and E1–E6 were from Dianchi Lake and Erhai Lake, respectively.

- l. 268-271: Which taxonomic order these genera belong to? Response: The revision has been made as follows: At genus level, Methanobacterium (within Methanobacteriales) had the greatest proportion, followed by Methanosaeta (within Methanosarcinales) and Methanoregula (within Methanomicrobiales)

- l. 303-304: Which clustering method was used here? Response: UPMGA

e. Discussion - l. 320-467: Here the structure chosen by the author for comparing their results with the ones from other previous studies looks a bit strange to me. First, they describe what was observed in other studies, and then, in a second sentence, they summarize what they found. This is confusing for the reader. I would do it the

other way around, i.e. “we observed this, which is consistent (or not) with previous observations”. Response: The authors appreciate the reviewer’s suggestions. The revision has been made as suggested.

- l. 320: In which kind of environment are you referring to? Response: The revision has been made as follows: The methanogenesis potential in freshwater lakes varied in a wide range

- l. 334-335: What do you mean by geological constitute, geographic regions and water type. Please be more specific and/or develop a little bit. Response: The revision has been made as follows: . . .including geological constitute (e.g. calcareous or not), geographical regions (Rinta et al., 2015) as well as water type (e.g. black water, clear wate) (Conrad et al., 2014). . .

- l. 339-341: What is the correlation between TOC (and other environmental parameters) and MPP, HMP and AMP? Response: Methane production might be related to several parameters (TOC, ORP and so on), and most of these parameters were also related to depth. So simple correlation would give misleading conclusion. Unluckily, data here was not enough for partial correlation. So correlation analysis was lacked here.

- l. 343-346: Did you find similar OTUs between these 2 lakes sediments? Response: Yes. The major OTUs (e.g. OTU1, OTU7) showed notable similarity.

- l. 357: Please develop. Response: The authors appreciate the reviewer’s suggestions. The revision has been made as suggested.

- l. 359-361: I don’t understand what the authors mean here. Response: The authors appreciate the reviewer’s suggestions. The revision has been made.

- l. 365-366: This is not clear. Should we conclude that shallow and eutrophic lakes are not stratified? Response: Yes, shallow lakes are not stratified.

- l. 370-371: Please develop here a bit more. Response: The authors appreciate the

reviewer’s suggestions. The revision has been made as suggested.

- l. 373-374: How is it possible to assess the abundance of methanotrophs with qPCR based on archaeal 16S primers? This makes no sense to me. Response: Some researchers have used 16S rRNA primers targeting certain orders of methanogens. The revision has been made as: The abundance of methanogens could be assessed using either order-specific archaeal 16S rRNA gene primers or mcrA gene primers.

- l. 377: Consistent with which results? Response: the results in the mentioned references.

- l. 379-380: What is the range of archaeal abundance described in the literature? Response: Response: The authors appreciate the reviewer’s suggestions. The revision has been made as: In the current study, the abundance of archaeal 16S rRNA gene was comparable to that reported in the literatures (about 1×107–2×109 copies/gDW in the top 20 cm) (Borrel et al., 2012; Zhu et al., 2012).

- l. 383: Different archaeal primers combinations were used for 16S quantification and sequencing. That could be an explanation for the different ratios observed. Response: The authors appreciate the reviewer’s suggestions. The revision has been made as: The result might suggest either the bias of amplification of mcrA gene or the difference between the two archaeal 16S rRNA primers used in the current study.

- l. 383-384: Do you mean that organisms can have multiple copies of mcrA? Or of 16S rRNA gene? Not really clear. Please develop and back-up with genomic data from literature. Response: The authors appreciate the reviewer’s suggestions. The revision has been made as: The result might suggest either the bias of amplification of mcrA gene or the difference between the two archaeal 16S rRNA primers used in the current study.

- l. 394-395: “but the change pattern: : :. was not clear”. I don’t understand it. Can you clarify? Response: The authors appreciate the reviewer’s suggestions. The revision

has been made as follows: but the mcrA gene diversity didn't show a regular change pattern

- l. 397-398: Can you point out the results you are discussing here? Response: The authors appreciate the reviewer's suggestions. The revision has been made as follows: In this current study, the sediment samples from Erhai Lake had slightly lower mcrA gene diversity than those from Dianchi Lake (Table 1).

- l. 418: Could you briefly introduce Taihu Lake? Response: As described in the reference, Lake Taihu is the third largest freshwater lake in China. It is s shallow eutrophic lake with an average depth of about 2 m.

- l. 420: "remarkably" is too strong here. I don't find this result very surprising... Response: The authors appreciate the reviewer's suggestions.The revision has been made as follows: ...archaeal community structure differed evidently in Dianchi Lake and Erhai Lake....

- l. 420: Please point out a figure to illustrate these findings. Response: The authors appreciate the reviewer's suggestions.The revision has been made as follows: In this study, the result of UniFrac-based cluster analysis (Figure 5a)...

- l. 412: Where do you see that depth is a key factor for archaeal community structure? Response: (If the referee means line 421 here.) Figure 5a showed that samples from upper layers (D1-D3, E1) were separated from others.

- l. 425-426: It seems that you are describing quantitative changes. But are they qualitative changes as well (at the genus level)? Response: We meant to describe qualitative changes. They qualitatively changed as well.

- l. 437-440: I would like to see this data. Response: Data was shown in Figure S3.

- l. 442: What do you mean by "the seventh"? The 7th to be discovered or the 7th most important? Not clear. Response: The revision has been made as follows: "and was the seventh order of methanogens to be found".

- l. 454-461: The congruency of phylogenetic trees does not depend on the relative proportion of the different OTUs. In fact, this has not impact. Do the 2 different trees have the same shape? Are the clusters the same between these 2 trees? Response: As reported by Luton (2002), the 2 different tree should have the sample shape and the same clusters.

f. Figures - Fig. 1 and 2: Flip over the axis to have the depth as vertical and descending axis. Precise which lake is eutrophic and which one is mesotrophic. Response: The authors appreciate the reviewer's suggestions. As suggested, the revision has been made.

- Fig. 1: Merge the 2 plots so it's easier to compare the results. Indicates MPP, AMP and HMP, and the AMP/HMP ratio. Response: The authors appreciate the reviewer's suggestions. As suggested, the revision has been made.

- Fig. 2: Use log scale for gene abundance. Response: The authors appreciate the reviewer's suggestions. As suggested, the revision has been made.

- Fig. 3: You should make this figure more informative. I suggest the following: you could start from the genus level. You indicate only genera that represent at least few % of the archaeal community (define a threshold) and merge the ones below this threshold in a category called "other <family name>". Then you do the same with the next taxonomic level (family), merging the small ones into the higher level (i.e. class), and so one. It's a little bit more of work to produce this figure, but at the end you have a plot that indicates the dominating groups, independently of the taxonomic level. An alternative is the Krona charts (https://sourceforge.net/p/krona/home/krona/). Response: The authors appreciate the reviewer's suggestions. It would be nice if we could use Krona charts here. However, it seems there is not enough space for 12 pie charts. We have considered using heatmap as well, but most genus are of low abundance, and heatmap couldn't show the structure well. Considering that we mainly focus on an overall composition and methanogens in the current study, other details are omitted in

the manuscript.

- Fig. 4: Highligh the E and D samples with a color code. Response: The authors appreciate the reviewer's suggestions. As suggested, the revision has been made.

- Fig. 5: The horizontal distances are not defined. Please use a color-code as well. Response: The authors appreciate the reviewer's suggestions. As suggested, the revision has been made.

3) Technical corrections a. Abstract - l. 25: "layer depth" is confusing. Please change it for "depth" throughout the whole document. Response: The authors appreciate the reviewer's suggestions. As suggested, the revision has been made.

b. Results - l. 191-193: These 2 first sentences are not clear. You should re-write them as follow: In this study, MPP varied remarkably with both lake and sediment depth except for the uppermost sediments layers which are remarkably similar between the two lakes Figure 1)." Response: As suggested, the revision has been made.

- l. 213: Remove "However". Response: As suggested, the revision has been made.

- l. 214: Rephrase "and showed an increase with depth: : :" Response: As suggested, the revision has been made.

- l. 220: Which depth these samples (D6 and E5) correspond to? Response: The revision has been made as follows: ...while the lowest one occurred in Dianchi Lake sample D6 (17-20cm, $2.5\pm0.3\times104$ copies/g dry weight) or Erhai Lake sample E5 (14-17 cm, $3.7\pm0.1\times104$ copies/g dry weight).

c. Discussion - l. 379: Please rephrase: ": : :the abundance of archaea: : :" Response: The revision has been made as follows: In the current study, the abundance of archaeal 16S rRNA gene

- l. 381: Specify here that this first ratio was calculated with qPCR results. Response: The revision has been made as follows: ...for each sample, the mcrA/16S ratio was

less than 3% according to the results of qPCR

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